Reprinted from

GENE

AN INTERNATIONAL JOURNAL ON
GENES AND GENOMES

*Sth*132I, a novel class-IIS restriction endonuclease of *Streptococcus thermophilus* ST132

M.T. Poch, G.A. Somkuti *, D.K.Y. Solaiman

GENE

AN INTERNATIONAL JOURNAL ON
GENES AND GENOMES

GENE/1996/$15.00

US mailing notice: GENE (ISSN 0378-1119) is published biweekly by Elsevier Science (Molenwerf 1, Postbus 211, 1000 AE Amsterdam, The Netherlands). Annual subscription price in the USA US $ 5069.00 (subject to change), including air speed delivery. Publications postage paid at Jamaica, NY 11431.
USA POSTMASTERS: Send address changes to Gene, Publications Expediting, Inc., 200 Meacham Avenue, Elmont, NY 11003.
Airfreight and mailing in the USA by Publications Expediting.

SUBSCRIPTION INFORMATION

Subscription price for 1996 (17 volumes) is Dfl. 8313.00 (US $ 5069.00), postage and handling included. The Dutch guilder price is definitive (the US $ price is subject to exchange rate fluctuations and is given only as a guide). For personal rates please apply to the publisher. Subscriptions are accepted on a prepaid basis only, unless different terms have been previously agreed upon.
Subscription orders can be entered only by calendar year (Jan–Dec) and should be addressed to:
Elsevier Science B.V., Journals Department, P.O. Box 211, 1000 AE Amsterdam (The Netherlands) Tel. +31 (20) 485 3642; Fax +31 (20) 485 3598, or to your usual subscription agent.
Postage and handling charges include surface delivery except to the following countries where air delivery via SAL (Surface Air Lift) mail is ensured; Argentina, Australia, Brazil, Canada, Hong Kong, India, Israel, Japan, Malaysia, Mexico, New Zealand, Pakistan, PR China, Singapore, South Africa, South Korea, Taiwan, Thailand, U.S.A. For all other countries airmail rates are available upon request.

Claims for missing issues should be made within six months of our publication (mailing) date, otherwise such claims cannot be honoured free of charge.

In the United States or Canada: for further information concerning this or any other Elsevier Science Publishers journal, contact: Elsevier Science Co., Inc., Journal Information Center, 655 Avenue of the Americas, New York, NY 10010 (U.S.A.). Tel. (212) 633-3750; Fax (212) 633-3990; Telex 420-643 AEP UI.

Founded in 1976 by Waclaw Szybalski

*Sth*132I, a novel class-IIS restriction endonuclease of *Streptococcus thermophilus* ST132

M.T. Poch, G.A. Somkuti *, D.K.Y. Solaiman

Abstract

The *Sth*132I restriction endonuclease (R.*Sth*132I) was detected in *Streptococcus thermophilus* ST132 and purified to near homogeneity by heparin Sepharose CL-6B affinity chromatography. Fragments from *Sth*132I digestion of plasmid DNA were subcloned into pUC19 in *Escherichia coli* DH5α and sequenced. Sequence analysis of inserts and their ligation junction sites revealed that *Sth*132I is a novel class-IIS restriction endonuclease, which recognizes the non-palindromic sequence
5′-CCCG$(N)_4$-3′
3′-GGGC$(N)_8$-5′.

Keywords: *Streptococcus thermophilus*; Restriction endonuclease; *Sth*132I

1. Introduction

Streptococcus thermophilus, an essential microbe in dairy food fermentations, is widely used in the production of a variety of food products. This industrially important species is a member of a diverse group of facultative anaerobes commonly referred to as lactic acid bacteria (LAB), which are used in various combinations to yield specific fermented foods. Recent genetic research on *S. thermophilus* has been focused on the characterization of indigenous plasmid DNAs Janzen et al., 1992; Hashiba et al., 1993), transformation techniques (Mercenier et al., 1987, 1988; Somkuti and Steinberg, 1988), carbohydrate metabolism (Herman and McKay, 1986; DeVos and Simons, 1988; Poolman et al., 1989, Poolman et al., 1990; Schroeder et al., 1991), amino acid metabolism (Yohda et al., 1991), promoter sequences (Slos et al., 1991; Constable and Mollet, 1994; cloning vectors (Solaiman et al., 1992; Solaiman and Somkuti, 1993), chromosome mapping (Roussel et al., 1994) and the expression of heterologous genes Solaiman and Somkuti, 1996).

In our laboratory, certain strains of *S. thermophilus* were observed to be refractory to transformation by electroporation with a variety of plasmids (Somkuti and Steinberg, 1988). This indicated the putative presence of restriction endonuclease (R.ENase) activity in these strains. Subsequently, our laboratory reported the occurrence and properties of R.*Sth*134I (Solaiman and Somkuti, 1990) and R.*Sth*117I (Solaiman and Somkuti, 1991) which were determined to be isoschizomers of R.*Hpa*II and R.*Eco*RII respectively. The presence of similar isoschizomers in different strains was later confirmed by others (Benbadis et al., 1991; Guimont et al., 1993).

Further screening of *S. thermophilus* strains in our collection has led to the discovery in *S. thermophilus* ST132 of a novel class-IIS R.ENase, R.*Sth*132I, which is the subject of this study.

Abbreviations: R.ENase, restriction endonuclease; LAB, lactic acid bacteria; LB agar, Luria-Bertani agar; R/M, restriction modification system(s); BSA, bovine serum albumin; TMM, Tris–$MgCl_2$-mercaptoethanol buffer; TM, Tris–mercaptoethanol buffer; EDTA, ethylenediaminotetracetate; AGE, agarose gel electrophoresis; SDS–PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; kb, kilobase(s); bp, base pair(s).

2. Experimental and discussion

*2.1. Isolation and purification of R.*Sth*132I*

The screening of *S. thermophilus* cultures with a rapid procedure (Poch and Somkuti, 1993), identified the plasmid-free strain ST132 as a putative carrier of R.ENase activity. Cell extracts of *S. thermophilus* ST132 were prepared from 1000-ml culture as previously described (Somkuti and Steinberg, 1988), and disintegrated by exposure for 1 h to mutanolysin (2 mg/ml, Sigma Chemical Co., St. Louis, MO) followed by sonic disruption. The centrifuged (12 000 × **g**) supernatant of the crude extract (approx. 15 mg/ml protein) was loaded on a 5-ml DEAE-cellulose (DE52) column, followed by washing with Tris (10 mM)–$MgCl_2$ (20 mM)–mercaptoethanol (10 mM) (TMM, pH 7.6) buffer until attainment of a nonabsorbing baseline at A_{280}. R.ENase activity was desorbed with a 25–300 mM linear gradient of KCl in TMM (20 ml) with active fractions collected at approx. 55–75 mM KCl. Nonspecific nucleases did not desorb until [KCl] reached approx. 115 mM. Fractions with R.ENase activity were pooled and further purified by heparin Sepharose CL-6B chromatography (Fig. 1A). The yield of purified R.*Sth*132I was 40 μg protein from an initial 12 mg in the crude extract. Only 40 units of enzyme were obtained per liter of cells. One enzyme unit was defined as the amount of protein required to digest 1 μg of phiX174 RF DNA after 1 h of incubation at 50°C in a 50 μl reaction volume. SDS–PAGE analysis of the final preparation of R.*Sth*132I showed essentially a single major band with an approx. 53.6 kDa molecular mass (Fig. 1B).

2.2. *Characterization of R.*Sth*132I*

Optimum conditions for *Sth*132I activity were determined with phiX174 or pER8 (Somkuti and Steinberg, 1986) as the DNA substrates. Similar to the two other R.ENases (R.*Sth*134I and R.*Sth*117I) described previously in *S. thermophilus* R.*Sth*132I had a requirement of at least 5 mM Mg^{2+} but no Na^+ or K^+ for activity. Furthermore, R.*Sth*132I also demonstrated a tolerance to salt concentrations of up to 250 mM Na^+ or K^+ in the reaction mixture and required an inactivation temperature greater than 60°C. R.ENase activity of *Sth*132I was determined to have an optimum pH of 7.5, with activity detectable between pH 6 and 9. The temperature optimum of R.*Sth*132I was 45°C, with measurable R.ENase activity from 37 to 55°C. In these respects, R.*Sth*132I was similar to R.*Sth*134I (Solaiman and Somkuti, 1990) but differed from R.*Sth*117I which displayed a nearly unchanged level of activity at temperatures between 30 and 50°C, a pH range from 5.4 to 8.3 (optimum at 6.5–7.0), and a loss of activity above 200 mM NaCl (Solaiman and Somkuti, 1991).

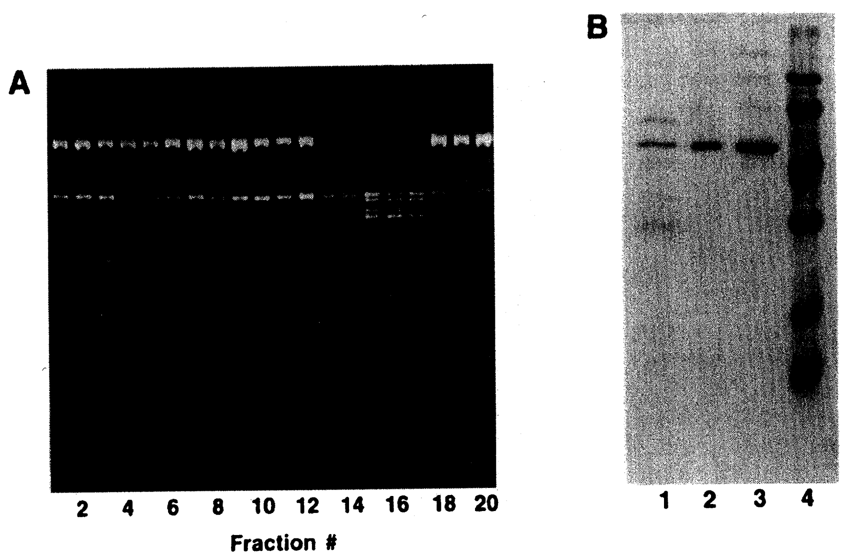


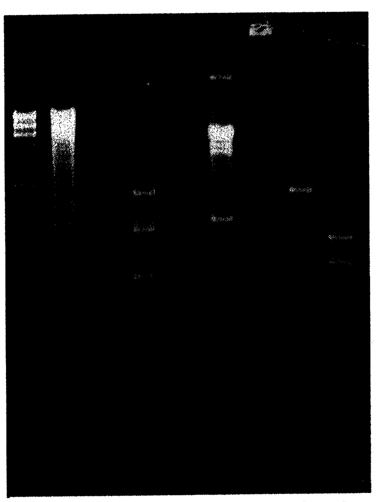
Fig. 1. Purification of *Sth*132I by heparin Sepharose CL-6B chromatography. (A) Fractions with R.ENase activity from the DE-52 anionic exchange column were pooled and loaded on a 1-ml activated heparin Sepharose CL-6B (Pharmacia LKB Biotechnology, Piscataway, NJ, USA) column equilibrated with Tris (10 mM)–mercaptoethanol (5 mM)–pH7.6 (TM) buffer and washed with same until A_{280} of eluant returned to baseline. Desorption of R.ENase activity was achieved with a 0.1–1.0 M linear gradient of NaCl in TM buffer (10 ml). Each fraction (0.5 ml) was tested by incubating a 10 μl sample with 0.4 μg of phi X-174 RF DNA, 100 μg bovine serum albumin, 2 μl of 10 × TMM buffer and sterile dH_2O in 20 μl total volume, at 45°C for 1.5 h. The reaction was stopped by the addition of 10 μl loading buffer (258 mM Tris–258 mM boric acid–7.5 mM EDTA, pH 8.2). Digests were developed in a horizontal 1.2% agarose gel slab for 3 h at 100 V followed by staining with ethidium bromide. R.*Sth*132I eluted at approx. 375 mM NaCl. Fractions with R.ENase activity were pooled and desalted on a Centricon-30 microconcentrator (Amicon, Beverly, MA, USA). The purified R.*Sth*132I was eluted with 200 μl of TM buffer, supplemented with 0.2 mM PMSF, and stored at −20°C. (B) SDS–PAGE analysis of purified R.*Sth*132I and various isolation intermediates. Samples (approx. 10 μg) containing the enzyme were developed in a 12.5% SDS–PAGE gel and stained with Coomassie brilliant blue (Laemmli, 1970). Lane 1: pooled fractions with R.ENase activity eluted from DE-52. Lane 2: R.*Sth*132I further purified on a heparin Sepharose CL-6B column. Lane 3: R.*Sth*132I isolated with a rapid minipreparative method (Poch and Somkuti, 1993). Lane 4: Standards (from top to bottom); phosphorylase *b* (94 kDa); bovine serum albumin (67 kDa); ovalbumin (43 kDa); carbonic anhydrase (30 kDa); soybean trypsin inhibitor (20.1 kDa) and α-lactalbumin (14.4 kDa).

R.*Sth*132I retained activity for several months when stored at 4°C in a buffer consisting of 10 mM Tris (pH 7.5)–0.1 mM EDTA–0.2 mM PMSF and 0.1% Triton X-100.

R.*Sth*132I digested lambda bacteriophage, T7-coliphage, adenovirus DNA, phiX174 RF DNA, SV40 DNA, pBR322, pUC18 and pER8 into several fragments (Fig. 2). Digestion patterns did not change appreciably beyond 3 h of incubation. As described later in the text, some of these bands were the results of incomplete digestion of the corresponding plasmid/DNA substrates. Of particular interest was the fragmentation pattern of pER8 which was later used for determining the site specificity of R.*Sth*132I (Fig. 2, lane 8). This cryptic plasmid from *S. thermophilus* ST108 was apparently digested into two major fragments (approx. 1.3 and 0.8 kb) but occasionally three smaller and less visible bands (approx. 60, 50 and 30 bp) were also detectable in agarose gels stained with ethidium bromide.

2.3. Determination of recognition site of R.Sth*132i*

Fragments generated from digesting pER8 with R.*Sth*132I were shotgun cloned (Sambrook et al., 1989) into the *Hin*cII site of pUC19 two ways: (1) by direct ligation assuming that R.*Sth*132I generated blunt-end termini, and (2) by prior treatment of pER8 fragments



1 2 3 4 5 6 7 8 9

Fig. 2. Restriction endonuclease activity of R.*Sth*132I with various substrates: AGE patterns of DNAs digested with R.*Sth*132I (substrate DNAs were purchased from New England Biolabs, Beverly, MA, USA); pER8 was prepared in our laboratory as previously described (Somkuti and Steinberg, 1986); R.ENases were products of Gibco BRL, Grand Island, NY, USA). Reaction conditions were the same as described in Fig. 1A. Lane 1:R.*Hin*dIII digest of lambda bacteriophage DNA (from top to bottom); 23 kb, 9.1 kb, 6.4 kb, 4.3 kb, 2.3 kb, 2.0 kb and 0.546 kb. Lanes 2–8: lambda bacteriophage DNA, adenovirus DNA, phi X174 RF DNA, pBR322, SV40 DNA, pUC18 and pER8 digested with R.*Sth*132I. Lane 9: R.*Hae*III digest of phiX174 DNA (from top to bottom); 1353 bp, 1078 bp, 872 bp, 603 bp and 306 bp.

with Klenow fragment (DNA polymerase I) or T4 DNA polymerase assuming that the enzyme generated cohesive ends. However, transformation of *Escherichia coli* DH5α was successful only with ligation products of pUC19 and Klenow-treated or T4 DNA polymerase-treated pER8 fragments confirming that R.*Sth*132I created cohesive termini. Plasmid analysis of transformants picked randomly from LB agar plates supplemented with 100 μg/ml ampicillin showed the presence of recombinant (pUCER) plasmids of three identifiable size classes. These plasmids (pUCER56, pUCER778 and pUCER1231) were purified and their respective ligation junction regions were examined by sequencing. The partial nucleic acid sequence showing the ligation junction region of pUCER56 is shown in Fig. 3A (italics). Sequence analysis comparing the insert sequence with that of pER8 showed that the 56-bp insert was located on the pER8 map at coordinates 835–890 (Fig. 3B, fragment A). Similar sequence analysis (Fig. 3B) localized the inserts in pUCER778 and pUCER1231 to map coordinates 919–1696 (fragment B) and 1754–890 (fragment C) of pER8, respectively. Sequence comparison of these ligation junction sites of pER8 did not reveal a consensus palindromic sequence characteristic of the recognition/cleavage site of the commonly known class-II restriction enzymes. However, careful examination of the sequences adjacent to these sites showed that a consensus sequence 5′CCCG3′ was located either upstream or downstream from the junction points (Fig. 3B). Sequence analysis of pER8 of *S. thermophilus* ST128 (2094 bp, our unpublished data) to locate this structure showed five possible sites on this plasmid, at coordinates 843C, 895C, 911, 1685 and 1746 (Fig. 4A), confirming the results of preliminary restriction analyses of pER8 with R.*Sth*132I (Fig. 2). Further, it was noted that the consensus sequence is situated 4- and 8-bp away from the junction points but showing no specific downstream base-pairing requirement (Fig. 4B). These results indicated that digestion with R.*Sth*132I leads to the generation of DNA fragments with 5′ protruding ends. Analysis of nucleic acid sequences retrieved from GenBank and EMBL databases programs showed varying number of 5′-CCCG-3′ recognition sites in other DNA substrates such as lambda bacteriophage (364), pBR322 (42), pUC19 (25), SV40 (4) and phiX174 (8), corroborating the results of earlier digestion experiments with R.*Sth*132I as shown in Fig. 2. Notable among these were the digestion patterns produced from phiX174 RF DNA and pER8. When digested with *Sth*132I, these substrates were predicted to produce eight (1, 89, 317, 493, 634, 895, 1418 and 1539 bp) and five (32, 52, 61, 774 and 1175 bp) fragments, respectively. Fig. 2 shows that phiX174 RF digests (lane 4) indeed contained the expected 634, 895, 1418 and 1539 bp bands. The digestion pattern of pER8 (Fig. 2, lane 8) showed the expected 774-bp and another, approx. 1300-bp partially

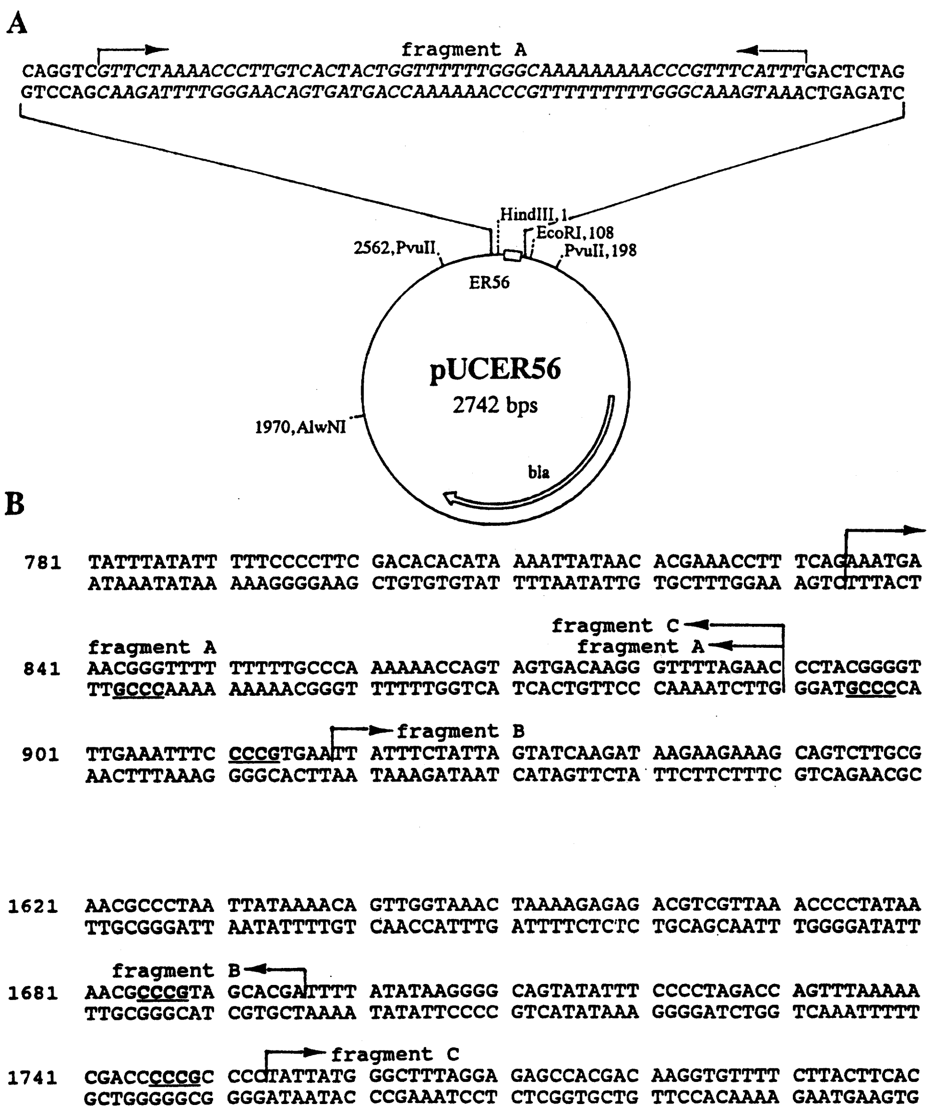
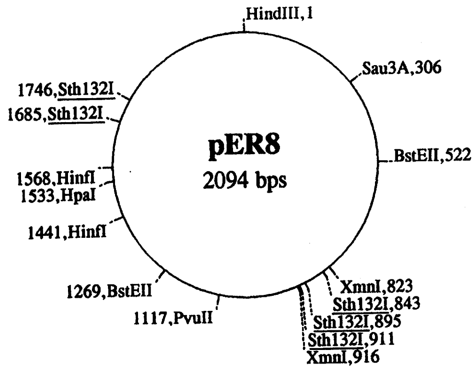


Fig. 3. (A) Partial nucleotide sequence of pUCER56. Sequence shown includes the ligation junctions of the 56-bp Klenow-fragment treated pER8 insert in the multiple cloning site of pUC19. Sequence was determined by the dideoxynucleotide chain termination method (Sanger et al., 1977) using AutoRead™ Sequencing Kit with M13 universal and M13 reverse primers, in an ALF.DNA Sequencer unit (Pharmacia Biotech, Piscataway, NJ, USA). (B) Partial sequence of pER8 showing ligation junctions of Klenow-treated 778 bp (B) and 1231 bp (C) fragments generated by R.*Sth*132I digestion.

digested band which apparently still included some of the predicted 32-, 52- and 61-bp fragments. Although occasionally seen as faint bands, the smaller fragments arising from the digestion of pER8 were usually below the level of detectability in ethidium bromide-stained agarose gels. The presence of bands larger than the longest restriction fragments expected suggested that digestion of both phiX174 RF and pER8 by R.*Sth*132I remained incomplete, possibly resulting from the varying sensitivity of the 5′-CCCG-3′ recognition site caused by differences in flanking nucleotide sequences.

The focus of research on R.ENases in the LAB group has been on phage resistance mechanisms involving restriction and modification (R/M) systems. Several laboratories have reported on R/M systems in lactococci (Fitzgerald et al., 1982; Chopin et al., 1984; Gautier and Chopin, 1987; Hill et al., 1989; Josephensen and Vogensen, 1989; Davis et al., 1993; O'Sullivan et al., 1995) and lactobacilli (Reyes-Gavilan et al., 1990). Data on R.ENases of lactic acid bacteria at the molecular level is limited to the lactococcal R.*Scr*FI (Fitzgerald et al., 1982) and R.*Lla*I (Hill et al., 1989; 1991; O'Sullivan et al., 1995) systems, and R.ENases of *S. thermophilus* Two other R.ENases reported to occur in *L. lactis* subsp. *cremoris*, R.*Lla*AI and R.*Lla*BI (Nyengaard et al., unpublished observations), were subsequently reclassified as isoschizomers of R.*Mbo*I and R.*Sfc*I, respectively (Roberts and Macelis, 1993). In the case of R.*Sth*132I, further research is needed to explore the potential role of this enzyme in R/M systems that

A



B

R.Sth132I Cleavage Sites in pER8

Coordinate position	Downstream Sequence
843C*	5'-CCCGTTTCATTT 3'-GGGCAAAGTAAA
895C*	5'-CCCGTAGGGTTC 3'-GGGCATCCCAAG
911	5'-CCCGTGAATTAT 3'-GGGCACTTAATA
1685	5'-CCCGTAGCACGA 3'-GGGCATCGTGCT
1746	5'-CCCGCCCCTATT 3'-GGGCGGGGATAA

Fig. 4. (A) Locations of the CCCG consensus sequence in pER8. (B) Variability in nucleotide base pairings downstream from CCCG sites in pER8. Arrows indicate cleavage points resulting in 5′ protruding ends; 'C' indicates the position of restriction sites on the complementary DNA strand.

may be operational in *S. thermophilus* or other members of the LAB group.

A search of the available literature failed to identify an isoschizomeric relationship between R.*Sth*132I and any of the known class-II or class-IIS R.ENase enzymes (Wilson, 1991; Roberts and Macelis, 1993). Therefore we concluded that R.*Sth*132I is a novel class-IIS R.ENase requiring an asymmetric and uninterrupted four-base non-palindromic recognition sequence,

5-'CCCG(N)$_4$

3-'GGGC(N)$_8$. It is also the first class-IIS restriction endonuclease to be characterized in *S. thermophilus*.

Acknowledgement

We acknowledge D.H. Steinberg for excellent technical expertise. Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

References

Benbadis, L., Garel, J.R., Hartley, D.L., 1991. Purification, properties and sequence specificity of *Sst*I, a new type II restriction endonuclease from *Streptococcus salivarius* subsp. *thermophilus*. Appl. Environ. Microbiol. 57, 3677–3678.

Chopin, A., Chopin, M.C., Moillo-Batt, A., Langella, P., 1984. Two plasmid-determined restriction and modification systems in *Streptococcus lactis*. Plasmid 11, 260–263.

Constable, A., Mollet, B., 1994. Isolation and characterization of promoter regions from *Streptococcus thermophilus*. FEMS Microbiol. Lett. 122, 85–90.

Davis, R., van der Lelie, D., Mercenier, A., Daly, C., Fitzgerald, G.F., 1993. *Scr*FI restriction modification system of *Lactococcus lactis* subsp. *cremoris* UC503: cloning and characterization of two *Scr*FI methylase genes. Appl. Environ. Microbiol. 59, 777–785.

DeVos, W.M., Simons, G., 1988. Molecular cloning of lactose genes in dairy lactic streptococci: the phospho-ß-galactosidase and ß-galactosidase genes and their expression products. Biochimie 70, 461–473.

Fitzgerald, G.F., Daly, C., Brown, L.R., Gingeras, T.R., 1982. *Scr*FI: a new sequence specific endonuclease from *Streptococcus cremoris*. Nucleic Acids Res. 10, 8171–8179.

Gautier, M., Chopin, M.C., 1987. Plasmid-determined systems for restriction modification activity and abortive infection in *Streptococcus cremoris*. Appl. Environ. Microbiol. 53, 923–927.

Guimont, C., Henry, P., Linden, G., 1993. Restriction/modification in *Streptococcus thermophilus*: isolation and characterization of a type II restriction endonuclease *Sth*455I. Appl. Environ. Microbiol. 39, 216–220.

Hashiba, H., Takiguchi, R., Joho, K., Aoyama, K., Hirota, T., 1993. Identification of the replication region of *Streptococcus thermophilus* No. 29 plasmid pST1. Biosci. Biotech. Res. 57, 1646–1649.

Herman, R.E., McKay, L.L., 1986. Cloning and expression of the β-galactosidase gene from *Streptococcus thermophilus* in *Escherichia coli*. Appl. Environ. Microbiol. 52, 45–50.

Hill, C., Pierce, K., Klaenhammer, T.R., The conjugative plasmid pTR2030 encodes two bacteriophage defense mechanisms in lactococci, restriction modification (R^+/M^+) and abortive infection (Hsp^+). 1989. Appl. Environ. Microbiol. 55, 2416–2419.

Hill, C., Miller, L.A., Klaenhammer, T.R., 1991. In vivo genetic exchange of a functional domain from a type II A methylase between lactococcal plasmid pTR2030 and a virulent bacteriophage. J. Bacteriol. 173, 4363–4370.

Janzen, T., Kleinschmidt, J., Neve, H., Geis, A., 1992. Sequencing and characterization of pST1, a cryptic plasmid from *Streptococcus thermophilus*. FEMS Microbiol. Lett. 95, 175–180.

Josephensen, J., Vogensen, F.K., 1989. Identification of three different plasmid-encoded restriction/modification systems in *Streptococcus lactis* subsp. *cremoris* W56. FEMS Microbiol. Lett. 59, 161–166.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.

Mercenier, A., Robert, C., Romero, D.A., Slos, P., Lemoine, Y., 1987. Transfection of *Streptococcus thermophilus* spheroplasts. In: Ferretti, J.J. and Curtiss III, R. (Eds.), Streptococcal Genetics. American Society for Microbiology, Washington, DC, 1987, p. 234.

Mercenier, A., Slos, P., Faelen, M., Lecocq, J.-P., 1988. Plasmid transduction in *Streptococcus thermophilus*. Mol. Gen. Genet. 212, 386

O'Sullivan, D.J., Zagula, K., Klaenhammer, T.R., 1995. In vivo restriction by *Lla*I is encoded by three genes, arranged in an operon with *lla*IM, on the conjugative *Lactococcus* plasmid pTR2030. J. Bacteriol. 177, 134–143.

Poch, M.T., Somkuti, G.A., 1993. Rapid screening of lactic acid bacteria for restriction endonuclease activity. Biotechnol. Tech. 7, 781–784.

Poolman, B., Roger, T.J., Mainzer, S.E., Schmidt, B.F., 1989. Lactose transport system of *Streptococcus thermophilus*: a hybrid protein with homology to the melibiose carrier and enzyme III of phosphoenolpyruvate-dependent phosphotransferase system. J. Bacteriol. 171, 244–253.

Poolman, B., Roger, T.J., Mainzer, S.E., Schmidt, B.F., 1990. Carbohydrate utilization in *Streptococcus thermophilus*: characterization of the genes for aldolase 1-epimerase (mutatrotase) and UDPglucose 4-epimerase. J. Bacteriol. 172, 4037–4047.

Reyes-Gavilan, C.G., Limsowtin, G.K.Y., Sechaud, L., Veaux, M., Accolas, J.P., 1990. Evidence for a plasmid-linked restriction–modification system in *Lactobacillus helveticus*. Appl. Environ. Microbiol. 56, 3412–3419.

Roberts, R.J., Macelis, D., 1993. REBASE-restriction enzymes and methylases. Nucleic Acids Res. 21, 3125–3137.

Roussel, Y., Pebay, M., Guedon, G., Simonet, J.M., Decaris, B., 1994. Physical and genetic map of *Streptococcus thermophilus* A054. J. Bacteriol. 176, 7413–7422.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sanger, F., Nicklen, S., Coulson, A.R., 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74, 5463–5467.

Schroeder, C.J., Robert, C., Lenzen, G., McKay, L.L., Mercenier, A., Analysis of the *lacZ* sequences from two *Streptococcus thermophilus* strains: comparison with the *Escherichia coli* and *Lactobacillus bulgaricus* β-galactosidase sequences. 1991. J. Gen. Microbiol. 137, 369–380.

Slos, P., Bourquin, J.C., Lemoine, Y., Mercenier, A., 1991. Isolation and characterization of chromosomal promoters of *Streptococcus salivarius* subsp. *thermophilus*. Appl. Environ. Microbiol. 57, 1333–3339.

Solaiman, D.K.Y., Somkuti, G.A., 1990. Isolation and characterization of a type II restriction endonuclease from *Streptococcus thermophilus*. FEMS Microbiol. Lett. 67, 261–266.

Solaiman, D.K.Y., Somkuti, G.A., 1991. A type II restriction endonuclease of *Streptococcus thermophilus* ST117. FEMS Microbiol. Lett. 80, 75–80.

Solaiman, D.K.Y., Somkuti, G.A., 1995. Expression of *cho*A and *mel*C operons by a *Streptococcus thermophilus* synthetic promoter in *Escherichia coli*. Appl. Environ. Microbiol. 43, 285–290.

Solaiman, D.K.Y., Somkuti, G.A., 1995. Expression of *Streptomyces mel*C and *cho*A genes by a cloned *Streptococcus thermophilus* promoter ST_{P2201}. J. Ind. Microbiol. 15, 39–44.

Solaiman, D.K., 1993. Y and Somkuti G.A., Shuttle vectors developed from *Streptococcus thermophilus* native plasmid. Plasmid 30, 67–78.

Solaiman, D.K.Y., Somkuti, G.A., 1996. Expression of a rhodococcal indigo gene in *Streptococcus thermophilus*. Biotechnol. Lett. 18, 19–24.

Solaiman, D.K.Y., Somkuti, G.A., Steinberg, D.H., 1992. Construction and characterization of shuttle plasmids for lactic acid bacteria and *Escherichia coli*. Plasmid 28, 25–36.

Somkuti, G.A., Steinberg, D.H., 1986. Distribution and analysis of plasmids in *Streptococcus thermophilus*. J. Ind. Microbiol. 1, 157–163.

Somkuti, G.A., Steinberg, D.H., 1988. Genetic transformation of *Streptococcus thermophilus* by electroporation. Biochimie 70, 579–585.

Somkuti, G.A., Steinberg, D.H., 1991. DNA-DNA hybridization analysis of *Streptococcus thermophilus* plasmids. FEMS Microbiol. Lett. 78, 271–276.

Somkuti, G.A., Solaiman, D.K.Y., Johnson, T.L., Steinberg, D.H., 1991. Transfer and expression of a *Streptomyces* cholesterol oxidase gene in *Streptococcus thermophilus*. Biotechnol. Appl. Biochem. 13, 238–245.

Somkuti, G.A., Solaiman, D.K.Y., Steinberg, D.H., 1993. Cloning of a tyrosinase gene in *Streptococcus thermophilus*. Biotechnol. Lett. 15, 773–778.

Somkuti, G.A., Solaiman, D.K.Y., Steinberg, D.H., 1995. Native promoter-plasmid vector system for heterologous cholesterol oxidase synthesis in *Streptococcus thermophilus*. Plasmid 33, 7–14.

Wilson, G.G., 1991. Organization of restriction-modification systems. Nucleic Acids Res. 19, 2539–2566.

Yohda, M., Okada, H., Kumagai, H., 1991. Molecular cloning and nucleotide sequencing of the aspartate racemase gene from lactic acid bacteria *Streptococcus thermophilus*. Biochim. Biophys. Acta 1089, 234–240.

Submission of papers

Full instructions to authors are to be found at the back of each issue. Please note the specific instructions regarding floppy disks. Please submit your paper to one of the journal's editors after consulting the editors' areas of expertise. The lists of editors and their fields are to be found at the front of each issue of the journal. Always check the editors' availability at the time of submission.

In the event that no appropriate editor can be identified, or for general queries regarding the submission of a paper or the reviewing process, please contact the Managing Editor:

Dr. A. Bernardi
Laboratoire de Génétique Moléculaire
Institut Jacques Monod, CNRS
Tour 43 (2e etage), 2 Place Jussieu
75000 Paris, France
Fax: +33 1 44277977
e-mail: abernard@ccr.jussieu.fr

Manuscript and proofs

Upon receipt of an accepted paper by the Publisher, a letter of acknowledgement will be sent by the Publisher to the corresponding author. Forms for the transfer of copyright and the ordering of offprints, extra to the 50 free offprints supplied per article, will be sent along with this acknowledgement letter. The corresponding author will later receive a proof of the article. Corrections (typesetting problems only) should be returned within 2 days of receipt to:

Gene
Log-in Department
Elsevier Science B.V.
P.O. Box 2759
1000 CT Amsterdam
The Netherlands
(Courier: Molenwerf 1
1014 AG Amsterdam
The Netherlands)
Fax: +31 20 485 3239

Publication

Questions pertaining to the publication of papers in issues may be addressed to

Issue Manager — Gene
P.O. Box 2759
1000 CT Amsterdam
The Netherlands
Fax: +31 20 485 2431
e-mail: g.mettam@elsevier.nl

Always quote 'Gene' and the article number in any correspondence